Fast Tissue Identification with Mass Spectrometry - A New Technology for In-vivo, In-situ Tissue Identification During Surgical Interventions

PhD theses

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INTRODUCTION, AIMS

In the past years a great revolution has begun in the science of medicine. The intuitive curing is altered by „stratified medicine" based on molecular diagnoses. The classical diseases are classified in smaller, but more homogenous groups based on molecular markers, and each group could be the target of special medicine(s). The aim is to reduce the unexpected side-effects in special cases and to filter out the false negative cases. The biomarkers, the target molecules of molecular diagnostics, are usually gained with large capacity scanning methods. These biomarkers are in general protein, metabolite or RNA-DNA molecules. However, recently specific and reproducible molecular signatures or fingerprints are also used as biomarkers, even if not all of the molecules have been identified precisely.

Our original goal was to create a fast and reliable method for the diagnosis of diseases in the central nervous system. We need a specific molecular marker or fingerprint for diagnosis. A number of methods are known nowadays as systems biology methods for finding molecular fingerprints. The advantage of these –omics methods (proteomics, genomics, lipidomics, metabolomics) is that all molecules of a complete system is analyzed, not only a specific molecule or a set of molecules, thus these techniques can be used for wide range marker or fingerprint scanning. The most recently used proteomics methods include 2 dimensional HPLC, gel-electrophoresis and mass spectrometry.

While studying Biology and working on my PhD, I also studied Computer Engineering, therefore I had wider and wider view on software engineering and mathematical analysis of data. My motivation was to use my qualifications in Biology, Mathematics and Informatics together to create and analyze huge systems biology data sets. That is how I started the development of a medical diagnostic tool.

The diagnostic tool is based on the surgical smoke – or in case of ultrasonic aspiration the waste solution – generated during surgery. This smoke or solution is lead away by a Teflon tube and analyzed with a mass spectrometer. Our observations suggest that the mass spectrometry lipid fingerprint of various tissue samples is different, thus many different tissue alterations – tumors, pathological alterations, inflammations – can be detected with this tool based on lipids analyzed by mass spectrometry. The most important feature of this tool is, that the tissue characterization takes place in less than 1 second(!), even during surgical

interventions. No sample preparation or tissue removing from the patient is necessary, the monitoring is directly on the living tissue surface, with a minimally invasive method, real-time. The surgeon can routinely use the accustomed tools (monopolar cutting device, bipolar forceps or ultrasonic aspirator) during surgery, it is not necessary to acquire new techniques, rebuild the theatre, or use any pretreatment on the patient.

The goal of my work was to create a new medical diagnostic tool based on routinely used surgical tools, focusing on application and the background of the data analysis (software, algorithm and database). After creating the first prototype, the continuous development of the tool using the experience gained from the theatre during surgical interventions, the validation of the method and the application to the identification of tumors were also my aims.

APPLIED METHODS AND SCIENTIFIC RESULTS

The aim of my PhD work was to develop a surgical tool for fast, real-time identification of different pathological tissue alterations, focusing on the separation of malignant tumors. Instead of using specific markers, I tried a systems biology approach including proteomics and lipidomics.

1. APPLICATION OF DIFFERENT METHODS IN PROTEOMICS AND LIPIDOMICS

The first method I used was 1-dimenional gel-electrophoresis, however it did not separate the proteins of the brain completely, thus only alteration in protein groups could be observed. A more precise sample preparation would be necessary, which would even increase the 3 day sample measurement time for diagnostics. 2-dimensional gel-electrophoresis combined with protein identification with mass spectrometry is an appropriate method for analyzing comprehensive systems, however even if all necessary equipments are accessible, the sample identification would take at least 4 days, which cannot be considered as fast diagnostics.

Our newly developed mass spectrometry ionization method termed as Rapid Evaporative Ionization Mass Spectrometry (REIMS) brought the break-through in the sample identification time. This technique is based only on mass spectrometry and no sample preparation is necessary. With the methods and algorithms developed by me, after building a validated database, the sample processing time decreases to 0.1 seconds i. e. 0.1 seconds after sampling, the result can be visualized. Compared to the previous techniques during direct mass spectrometry without sample preparation, the classification of tissues is based on mainly lipid components.

This is how I ended up from electrophoresis based protein analysis to mass spectrometry based lipid fingerprint analysis during my research.

My results: testing of 1-dimensional gel-electrophoresis, 2-dimensional gel-electrophoresis and techniques based on mass spectrometry only.

2. DEVELOPMENT OF FAST TISSUE DIAGNOSTICAL METHOD USING MASS SPECTROMETRY

Rapid Evaporative Ionization Mass Spectrometry, combined with different surgical tools without any sample preparation, is a suitable method for membrane lipid distribution based organ and tissue identification. The technique can be used for identification of different (cancerous) pathological alterations when combined with surgical laser, electrosurgical tools (monopolar electrode) and bipolar forceps. In addition, tissue debris analysis generated by ultrasonic aspirator is also possible, and the resulting spectrum is also unique for different tissue types.

After further development of our REIMS combined method, our previous expectations were far exceeded. Not only healthy and cancerous tissue could be separated with my algorithm, but the tumors could be classified and primary tumors could be pointed out from sampling the metastasis. The different layers of intestinal wall, and various not cancerous pathological alterations can be also separated (benign alteration, inflammation, etc.).

Surgical tools combined

- monopolar handpiece – general surgery
- bipolar forceps – neurosurgery
- ultrasonic aspirator – liver- and neurosurgery
- medical laser – various
- endoscopic snare – gastroenterology
- laparoscopic interventions – general surgery

My results: combining monopolar handpiece, bipolar forceps, endoscopic snare and laparoscopic tool with intelligent surgical tool.

Used algorithm and supplemental material (the whole chapter is my result)

- background: PCA + LDA, component number: 60
- preprocessing of spectra: normalization, binning, averaging for database building and classification
- defining “outliers”, probability of classification
- supplemental algorithms: searching for appropriate marker peaks and monitoring them

Database and software:

- Oracle based database
- C# based software, SQL based algorithms. Two separate software, one for database management the other for data collection and real-time classification

My results: software planning, testing and uploading all data to the completed database. Building and testing PCA-LDA models.

3. IN-VIVO, IN-SITU TISSUE IDENTIFICATION IN THE THEATRE

During my research, I worked on the application of a novel mass spectrometry based tissue identification method. My main tasks were to insure the theoretical background and software and to start using the application in practice. I built a data processing method based on known algorithms containing personal additions. As a result, I could prove to the surgeons and through several publications, to the scientific community, that an in-vivo cancer surgical diagnostic tool, based on simple chemical information, can give relevant new information to the surgeons in the theatre. The first developed prototypes are already in placed out to different Clinics.

Surgical fields tested and combined application:

- Liver surgery: separation of cancerous and healthy tissue, identification of primary tumor from metastasis
- Lungs and corresponding lymphoid glands: separation of healthy tissue and different lung cancers, analyzing and identifying metastases in local lymphoid glands
- Colon and rectum surgery: database building for endoscopic measurements, unknown tissue identification during surgical interventions, healthy/cancerous tissue separation
- Stomach surgery: separation of healthy/cancerous tissue, unknown tissue identification, analysis of corresponding lymphoid glands
- Breast cancer surgery: separation of cancerous tissue from surrounding tissue, analysis of corresponding lymphoid glands
- Neurosurgery: separation of healthy/cancerous tissue, identification of different brain tumors, primary tumor localization from brain metastases

I created the setup for data collection, and all surgical fields were tested under my supervision.

SUMMARY

The work presented in my thesis has many potential in in-vivo diagnostics during surgical interventions. One of the most important applications is during primary brain tumor surgery, as the surgeon still has to rely partially on own experience and judgment when identifying the tumor margin. An objective diagnostic tool, which could assure the operating surgeon that the surrounding tissue does not contain any cancerous cells, or contrary, gives a warning that it still contains parts of the tumor, could be very helpful in brain surgery. A number of arguments can be advanced in favor of an in-vivo diagnostic tool in general surgery also (breast, liver, thyreoid gland surgery and any complications), not to mention the fact that the knowledge of several thousand pathologists can be combined in our database.

In conclusion, I think a REIMS based diagnostic tool would be great progress in cancer surgery. It could help the precise work, the on-table decision making of the surgeon and the survival of the patient.

Related publications

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Other publications:

7. E. M. Szego, K. Barabas, **J. Balog**, N. Szilagyi, K. S. Korach, G. Juhasz, I. M. Abraham Estrogen induces estrogen receptor alpha-dependent cAMP response element-binding protein phosphorylation via mitogen activated protein kinase pathway in basal forebrain cholinergic neurons in vivo. Journal of Neuroscience, 2006, 26(15), 4104-4110.
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All IF ≈ **44.648**

Oral presentations:

1. New Tech Meetup presentation – New Tech Meetup birthday & Pecha Kucha Night
2010.02.27. Budapest
Balog Júlia: – *Intelligent Surgical Tool - in-vivo, on-line oncological diagnostics in surgery (Hungarian)*

2. MTA Chemometrics and MKE QSAR scientific sessions
2010.04.29-30. Szeged,
Balog Júlia, Lopata Antal, Szaniszló Tamás, Takáts Zoltán: *Intelligent Surgical Tool – Classification of Healthy and Cancerous Tissue using Chemometric Methods (Hungarian)*

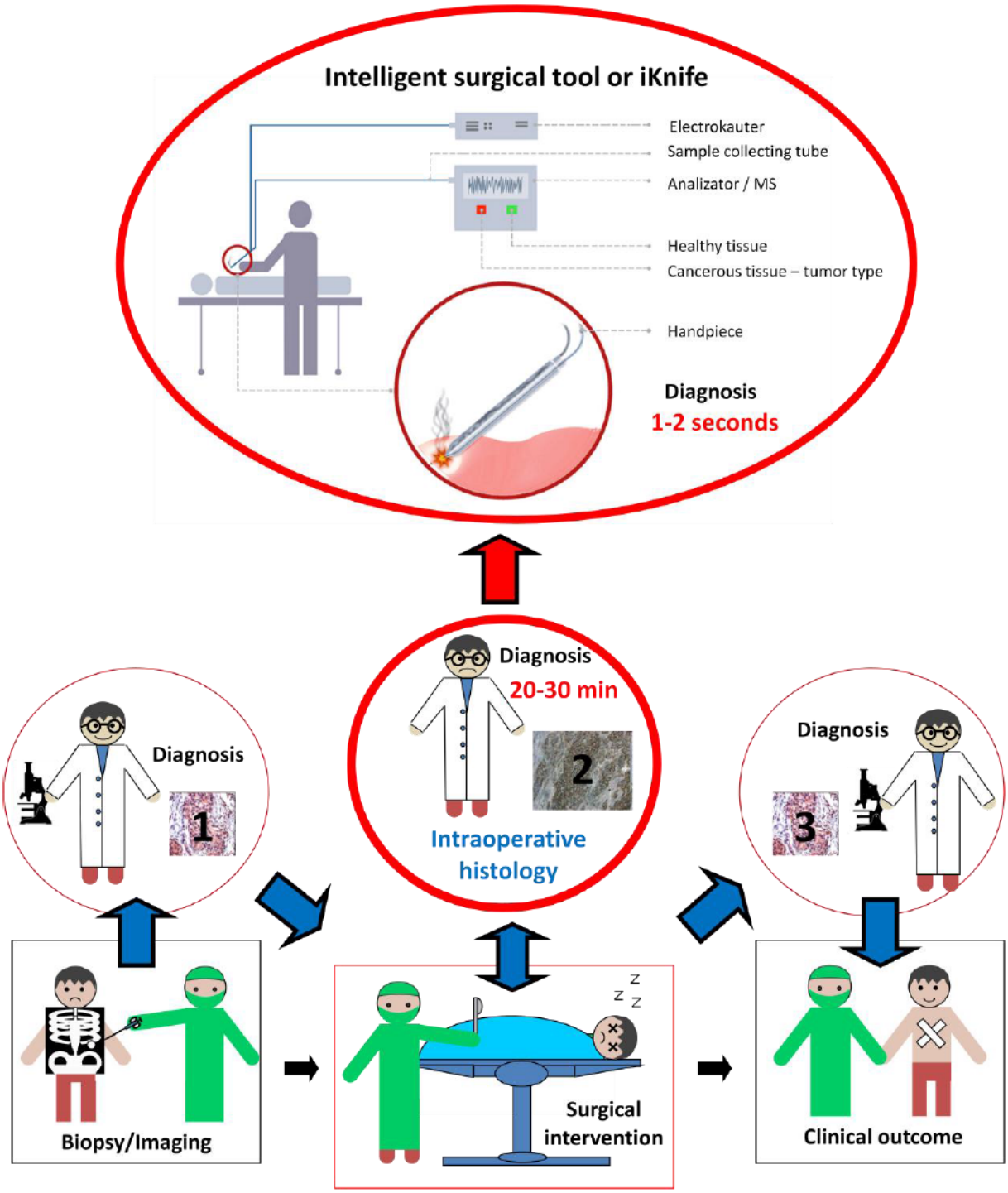
3. 58th ASMS Conference on Mass Spectrometry
2010.05.23-27. Salt Lake City Utah, USA
Julia Balog; Tamas Szaniszlo; Daniel Szalay; Lajos Godorhazy; Laszlo Sasi Szabo; Karl C Schaefer; Miklos Toth; Zoltan Takats: *Intraoperative Identification of Malignant Gastrointestinal Tumors and Proximal Metastases by Rapid Evaporative Ionization Mass Spectrometry*

Poszterek:

1. 59th ASMS Conference on Mass Spectrometry
   2011.06.05-09. Denver, Colorado, USA
   Julia Balog; Karl C Schaefer; Tamas Szaniszlo; Stefanie Gerbig; Zoltan Takats *Real-time interpretation of data during MS-guided surgical interventions*

2. 60th ASMS Conference on Mass Spectrometry
   2012.05.20-24. Vancouver, BC, Canada
   Julia Balog, Laszlo Sasi-Szabo, Zoltan Takats. *Improvement of real-time statistical analysis using tumor margins and tumor surrounding regions.*

3. Semmelweis Symposium 2012
   2012.11.09-10. Budapest, Hungary
   Julia Balog, Tamas Szaniszlo, Geza Mezey, Laszlo Bognar, Zoltan Takats. *Real-time tissue identification in the neurosurgical theatre.*


Intelligent surgical tool or iKnife
Electrokauter
Sample collecting tube
Analizator / MS
Healthy tissue
Cancerous tissue – tumor type
Handpiece
Diagnosis
1-2 seconds
Diagnosis
20-30 min
2
Intraoperative histology
Diagnosis
1
Diagnosis
3
Biopsy/Imaging
Surgical intervention
Clinical outcome